



# Analysis of Differences *Tumor Necrosis Factor- $\alpha$* (TNF- $\alpha$ ) Levels in Pregnant Women with Preeclampsia in the Bugis, Makassar, Mandar, and Toraja Tribes

Putri Kusuma Pandin<sup>1</sup>, Muh Nasrum Massi<sup>2</sup>, Sitti Wahyuni<sup>3</sup>, Prihantono<sup>4</sup>, Yuyun Widaningsih<sup>5</sup>

<sup>1</sup>Department of Midwifery, Graduate School, Hasanuddin University, Indonesia; <sup>2</sup>Department of Microbiology, Faculty of Medicine, Hasanuddin University, Indonesia; <sup>3</sup>Department of Parasitology, Faculty of Medicine, Hasanuddin University, Indonesia; <sup>4</sup>Department of Oncology, Faculty of Medicine, Hasanuddin University, Indonesia; <sup>5</sup>Department of Pathology Clinic, Faculty of Medicine, Hasanuddin University, Indonesia.

## ABSTRACT

**Objective:** This study aims to determine differences in levels of Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) in pregnant women with preeclampsia in the Bugis, Makassar, Mandar, and Toraja tribes so that they can be used as a basis for initial consideration to detect tribal variations as a risk factor for preeclampsia (PE).

**Method:** This study is an observational a case-control design conducted in March-July 2020 in several health centers and hospitals at Lasinrang. The number of samples used in this study was 88 people, 44 pregnant women with preeclampsia, and 44 normotensive pregnant women. The criteria of the sample in this study were pregnant women >20 weeks with a diagnosis of preeclampsia with blood pressure  $\geq 140 / 90$  mmHg and were not experiencing other pregnancy complications. Data collected includes age, parity, and Body Mass Index (BMI). TNF- $\alpha$  levels were assessed using the Human TNF- $\alpha$  ELISA Kit, Bioassay Technology Laboratory.

**Results:** There were significant differences in TNF- $\alpha$  levels in preeclampsia pregnant women where the highest TNF- $\alpha$  levels of pregnant women preeclampsia were in Makassar ( $181.12 \pm 36.27$  Pg/ml) while the Mandar tribe with the lowest TNF- $\alpha$  levels (Makassar  $143.30 \pm 22.74$  Pg/ml). While differences in TNF- $\alpha$  levels in preeclampsia and normotensive pregnant women in each tribe found significant results in the Makassar tribe ( $p < 0.001$ ), and Toraja ( $p = 0.028$ ), in the Bugis and Mandar tribes there were no significant results. ( $p > 0.05$ ).

**Conclusion:** There are differences in TNF- $\alpha$  levels in preeclampsia pregnant women and normotensive in the Makassar and Toraja tribes. So that tribal variation can be considered as a risk factor for preeclampsia pregnant women.

**Key Words:** Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), Preeclampsia, Tribe, Makassar, Mandar, Toraja, Bugis

## INTRODUCTION

Preeclampsia (PE) is the most common disorder in pregnancy and is one of the leading causes of maternal and fetal morbidity and mortality. PE affects 1-10% of pregnant women worldwide and is characterized by high blood pressure and proteinuria that occur after 20 weeks' gestation.<sup>1</sup> The aetiology of PE is not yet fully understood, but there is a theory that says that the placenta is closely related to the pathogenesis of PE. This relates to disorders between blood vessels and anti-angiogenic responses.<sup>2</sup> Another hypothesis

explains that the aetiology of PE is related to the immune response. Cytokines are substances that may be involved in the pathogenesis of PE. Type 1 cytokine receptors (interleukin-2), and type 2 cytokine receptors (interferon and alpha necrosis tumours) are generally produced in inflammatory processes caused by PE. Some previous studies have even shown variations in cytokine levels in women with PE even though the pathogenesis in this disease is still controversial.<sup>3</sup>

Cytokines are divided into 6 groups: interleukin, interferon, Colony-Stimulating Factor, Tumor Necrosis Factor,

### Corresponding Author:

**Putri Kusuma Pandina**, Department of Midwifery, Graduate School, Hasanuddin University, Indonesia;  
Email: [pandinpk18p@student.unhas.ac.id](mailto:pandinpk18p@student.unhas.ac.id); [pandinkusumaputri@gmail.com](mailto:pandinkusumaputri@gmail.com)

ISSN: 2231-2196 (Print)

ISSN: 0975-5241 (Online)

Received: 18.08.2020

Revised: 12.09.2020

Accepted: 20.10.2020

Published: 24.11.2020

Growth Factor, and Chemokine. Several types of cytokines have been shown to increase in preeclampsia and may be used as a marker of the diagnosis of preeclampsia. Inflammatory cytokines, such as IL-6 and TNF- $\alpha$ , are reported to be increased in preeclampsia.<sup>4</sup> *Tumour Necrosis Factor- $\alpha$*  (TNF- $\alpha$ ) is a proinflammatory cytokine that can exert various effects on various cells.<sup>5</sup> Hypoxia triggers the production of TNF- $\alpha$  which has a cytotoxic effect on endothelial cell blood vessels, placental trophoblast cells. This induces apoptosis and inhibits trophoblast cell proliferation, which causes vasoconstriction. TNF- $\alpha$  levels in the maternal circulation increase before the clinical manifestations of preeclampsia.<sup>6</sup> A study conducted in 2019 showed TNF- $\alpha$  levels were significantly increased ( $p < 0.05$ ) in women with preeclampsia compared to women with normal pregnancies.<sup>1</sup>

Another risk factor commonly associated with PE is ethnicity. In 2012, some studies showed Turkish and Moroccan women had a lower risk of PE compared to women from ethnic Dutch<sup>7</sup>. Another study in 2016 showed that black women had a higher percentage of PE development compared to white women.<sup>8</sup>

This study was conducted to analyze differences in TNF- $\alpha$  levels in pregnant women with PE in the Bugis, Makassar, Mandar, and Toraja tribes as a prognosis and prevention efforts for PE.

## MATERIALS AND METHODS

### Research sites

This research was conducted in March-July 2020 and has received an ethical approval recommendation with protocol number UH20020139. This research was conducted at the Hasanuddin University Hospital, Health center Jumpandang Baru, Health center Barabaraya, Health center Makale, Elim Toraja Utara hospital, Lakipadada Tana Toraja Regional Hospital, Totoli Health Center, Majene Public Hospital, Mattirobulu Health Center, and Lasinrang Pinrang General Hospital.

### Data types and sources

Data collected from respondents are demographic data (age, BMI before pregnancy, and history of PE). Data were taken from pregnant women of gestational age  $> 20$  weeks with a diagnosis of PE without comorbidities.

### Data collection technique

Demographic data were collected using a questionnaire through the method of direct interviews with respondents.

Meanwhile, for blood sampling, researchers were assisted by laboratory personnel at the Health center and research hospitals. The collected samples were then centrifuged and stored in the refrigerator at  $-20^{\circ}\text{C}$ . After all the samples were met, TNF- $\alpha$  levels were examined using the Human TNF- $\alpha$  ELISA Kit, Bioassay Technology Laboratory at Hasanuddin University Medical Research Center (HUM-RC) Laboratory. Data were analyzed using the Chi-Square test, the Shapiro-Wilk test, the Kruskal-Wallis test, and the Mann-Whitney test.

## RESULTS

Data shows that three characteristics, age, parity, and BMI among cases and control groups are almost similar. It's just that the characteristics of BMI in the 'normal' category, data shows total respondents of the control group highest than the case group (Table 1).

The results of the analysis showed that differences in TNF- $\alpha$  levels between PE pregnant women and normotension significant in Makassar and Toraja tribes. The mean rank of PE pregnant women in the Makassar tribe was 16.82, lower than the normotensive pregnant women 6.18 ( $p < 0.001$ ). Similarly, the Toraja tribes, the mean rank of PE pregnant women was 14.55, also lower than the normotensive pregnant women 8.45 ( $p = 0.028$ ). On the other hand, Bugis and Mandar tribes also show differences in TNF- $\alpha$  levels of PE pregnant women with normotensive pregnant women, but not as significant as the Makassar and Toraja tribes (Table 2).

**Table 1: Demographic Characteristics of Respondents**

Characteristics	Preeclampsia (n = 44)	Normal (n = 44)	p-value
<b>Age n (%)</b>			
High risk	7 (46.7)	8 (53.3)	0.500 *
Low risk	37 (50.7)	36 (49.3)	
<b>Parity n (%)</b>			
Primigravida	14 (51.9)	13 (48.1)	0.500 *
Multigravida	30 (49.2)	31 (50.8)	
<b>BMI n (%)</b>			
Underweight	0 (0)	1 (100)	0.005 *
Normal	8 (30.8)	18 (69.2)	
Overweight	21 (48.8)	22 (51.2)	
Obesity	15 (83.5)	3 (16.7)	

\*chi-square test

**Table 2: Differences TNF- $\alpha$  levels between preeclampsia pregnant women and normotensive pregnant women in the Bugis, Makassar, Mandar, and Toraja tribes (n = 88).**

Tribes	Preeclampsia Status	TNF- $\alpha$ Levels		p-value*
		Mean rank	Sum of rank (Pg/ml)	
Makassar	Preeclampsia	16.82	185.00	<0.001
	Normotension	6.18	68.00	
Bugis	Preeclampsia	12.64	139.00	0.438
	Normotension	10.36	114.00	
Mandar	Preeclampsia	13.18	145.00	0.243
	Normotension	9.82	108.00	
Toraja	Preeclampsia	14.55	160.00	0.028
	Normotension	8.45	93.00	

\*ujMann-Whitney test

## DISCUSSION

Based on statistical tests in table 1 shows that age and parity did not significantly influence the incidence of PE. Meanwhile, the test results for BMI showed a value of  $p = 0.005$  which means BMI influences the incidence of PE, there were 43 overweight people (48.9%), and 18 obese people (20.5%). A strong correlation was found between an increase in BMI and the risk of preeclampsia. The risk increased twofold for mothers with a BMI category  $> 25 \text{ kg/m}^2$ , and almost three times for mothers with a BMI category  $> 30 \text{ kg/m}^2$ . The increased risk affects not only white and African American women but also women of other ethnicities around the world.<sup>9</sup> Someone in the obese category shows metabolic syndrome which is characterized by insulin resistance with an excessive flux of fatty acids that contribute to disturbing the immunological balance.<sup>10</sup> On the other hand, metabolic syndrome is associated with endothelial dysfunction. Theory suggests that endothelial dysfunction, increased vascular permeability, and increased levels of circulating endothelial cell markers play an important role in the pathogenesis of preeclampsia.<sup>11</sup>

The results of the statistical test showed that from the four tribes, Makassar and Toraja ethnic groups had a significant difference in levels of TNF- $\alpha$ , with p-values  $<0.001$  and  $0.028$ , respectively. The results of this study are in line with the previous study which showed that preeclamptic pregnant women had a high pro-inflammatory cytokine response, while the anti-inflammatory response decreased. The higher levels of proinflammatory cytokines in the PE group were TNF- $\alpha$  and IL-15, while IL-10 had lower levels.<sup>2</sup> There is also a study that shows the same thing, that TNF- $\alpha$  levels were found to be significantly increased in preeclamptic pregnant women compared to normotensive pregnant women ( $p = 0.04$ ).<sup>12</sup> Another study that is in line with the results

of this study is a study conducted by Szarka et al. which showed TNF- $\alpha$  was significantly different in PE pregnant women with normotensive pregnant women ( $p < 0.001$ ).<sup>13</sup> TNF- $\alpha$  is a pro-inflammatory cytokine that plays a role in regulating many aspects of macrophage function. TNF- $\alpha$  is released immediately in conditions of trauma, infection, or exposure to bacteria. This proinflammatory cytokine has proven to be one of the most numerous early mediators in the inflamed tissue and is considered a “master regulator” of pro-inflammatory cytokine production.<sup>14</sup> TNF- $\alpha$  plays an important role in the pathogenesis of preeclampsia. There is the fact that higher circulating TNF-alpha levels are observed in preeclampsia than in gestational hypertension, suggesting an association with disease severity.<sup>12</sup> Inflammation is part of the complex biological response of the vascular network to harmful stimuli. The progressive destruction of tissue will endanger the survival of the organism. The inflammatory response is usually accompanied by an increase in the concentration of proinflammatory cytokines, an acute-phase protein, and may involve endothelial activation. Hypoxia promotes the overproduction of TNF- $\alpha$ , a proinflammatory cytokine factor, and has a potent cytotoxic effect on vascular endothelial cells, placental trophoblast cells. It induces apoptosis, inhibits trophoblast cell proliferation, increases endothelial expression of platelet-derived growth factor, endothelin-1, and plasminogen-1 activator inhibitor, all of which are associated with vasoconstriction<sup>6</sup>. Besides, a poorly perfused and hypoxic placenta is thought to synthesize an increased vasoactive factor, namely TNF-alpha. This increase can lead to endothelial dysfunction by reducing biologically available nitric oxide and increasing reactive oxygen species and endothelin-1 which in turn leads to altered renal function, decreased renal pressure natriuresis, increased total peripheral resistance, and hypertension. The ischemic placenta leads to endothelial cell activation/dysfunction and increased TNF-alpha synthesis which leads to induced structural and functional changes in endothelial cells leading to preeclampsia through TNF-alpha regulation of endothelin-1 gene transcription.<sup>12</sup>

The difference in TNF- $\alpha$  levels is influenced by several factors, severe infections can trigger the production of large amounts of TNF which cause systemic reactions, age, and ethnicity, infectious diseases, autoimmune, obesity, habit. smoking and alcohol consumption, high activity levels, history of oral contraceptives, parity, and advanced age at first pregnancy.<sup>15-17</sup> In this study, it was found that from four tribes, Makassar and Toraja had significant differences in levels of TNF- $\alpha$  levels of pregnant women with PE and normotension. Makassar and Toraja are regencies in South Sulawesi with different geographical locations, as well as air temperatures and sample characteristics. The geographical conditions of Makassar City, based on the altitude, vary between 0-25 meters above sea level, with an air temperature of  $20^{\circ}$ -  $32^{\circ}\text{C}$ . While Tana Toraja Regency is a plateau with

altitudes ranging from 300 -> 2,889 meters above sea level. The temperature in Tana Toraja ranges from 16<sup>0</sup> - 28<sup>0</sup>C.<sup>18</sup> A study conducted by Palmer et al. showed that in general, the incidence of preeclampsia was more common in the highlands ( $\pm$  3100m) ( $p < 0.002$ ).<sup>19</sup> Highland dwellings increase the risk of experiencing preeclampsia 3.6 times. There is also research conducted by Coussons-Read et al. showing that TNF- $\alpha$  levels were found to be significantly increased in pregnant women who live in the highlands compared to pregnant women who live in the lowlands ( $p < 0.045$ ).<sup>20</sup> This is related to the theory that pregnant women who live in the highlands are more prone to placental hypoxia caused by a lack of oxygen supply, which in turn causes oxidative stress and endothelial dysfunction, vasoconstriction occurs and leads to increased blood pressure.<sup>21</sup>

The difference in terms of air temperature in Makassar and Toraja was also discussed by Shahgheibi et al. in his research which concluded that the incidence of preeclampsia was higher in winter than in summer. Temperature changes affect plasma volume, in cold weather can cause vasospasm that can trigger the development of pathogenesis from preeclampsia.<sup>22</sup> Another relationship is explained Madhu et al. in their research that the risk of preeclampsia increases in winter ( $p < 0.001$ ) due to vitamin D deficiency. Kasitriol (the active form of vitamin D) functions as a hormone that regulates gene function in the implantation process. Vitamin deficiency can lead to abnormal implantations that trigger an inflammatory response, which is the precursor to the development of preeclampsia.<sup>23</sup>

## CONCLUSION

This study concluded that there are differences in TNF- $\alpha$  levels in preeclampsia pregnant women and normotensive in the Makassar and Toraja tribes. So that tribal variation can be considered as a risk factor for preeclampsia pregnant women.

## REFERENCES

- Ahmed MA, Alqosaibi AI, Mohamed MA, Soliman MG. Evaluation of Some Cytokines and Gene Expressions in Pre-eclampsia. *Pak J Biol Sci*. 2019;22 (3): 14.
- Fathollah K, Samira R, Amir BH, Reza M, Nesa R, Hosseinali I, et al. Serum levels of tumor necrosis factor- $\alpha$ , interleukin-15 and interleukin-10 in patients with pre-eclampsia in comparison with normotensive pregnant women. *Iran J Nurs Midwifery Res* 2013;18(6): 463–466.
- Sarengat M, Ganie RA, Lumbanraja SN. Levels of interleukin-6 and tumor necrosis factor-alpha in pregnant patients with preeclampsia and patients with normal pregnancy. *Indones J Clin Pathology Med Lab* 2019;25 (2):1.
- Anom IG, Kusuma AA. High Level of Tumor Necrosis Factor (TNF)- $\alpha$  is a Risk Factor for Preeclampsia. *Indones J Obst Gynecol* 2012;36(3):107-11.
- Azizeh FY, Raghupathy RG. Tumor Necrosis Factor- $\alpha$  and Pregnancy Complications: A Prospective Study. *Med Princ Pract*. 2014;24:165-70.
- Padmini E, Lavanya D. HIF-1 $\alpha$ , TNF- $\alpha$  and HO-1 Modulation in Placental Explants during Preeclampsia. *Int J Res Chem Environ* 2014;4(2):85-92.
- Bouthoorn SH, Gaillard R, Steegers EAP, Hofman A, Jaddoe VWV, Van Lenthe FJ, et al. Ethnic differences in blood pressure and hypertensive complications during pregnancy the generation R study. *Hypertension* 2012;60(1):198-205.
- Wangi JI, Dilmohamed A, Pierre Y. Difference In The Trend of Preeclampsia Between Black Women and White Women Living In The Uk. *IOSR J Pharm* 2016;6(2):62-73.
- Lopez-Jaramillo P, Barajas J, Rueda-Quirano SM, Lopez-Lopez C, Felix C. Obesity and Preeclampsia: Common Pathophysiological Mechanisms. *Front Physiol* 2018;9:1-10.
- Robillard PY, Dekker G, Scioscia M, Bonsante F, Iacobelli S, Boukerrou M, et al. Increased BMI has a linear association with late-onset preeclampsia: A population-based study. *PLoS One* 2019;14(10):1-14.
- Poorolajal J, Jenabi E. The association between body mass index and preeclampsia: a meta-analysis. *J Matern Neonatal Med* 2016;29(22):3670-6.
- Vitoratos N, Economou E, Iavazzo C, Panoulis K, Creatsas G. Maternal Serum Levels of TNF-Alpha and IL-6 Long after Delivery in Preeclamptic and Normotensive Pregnant Women. *Hindawi Publ Corp Mediat Inflamm*. 2010;2010: 908649.
- Szarka A, Rigó J, Lázár L, Beko G, Molvarec A. Circulating cytokines, chemokines and adhesion molecules in normal pregnancy and preeclampsia determined by multiplex suspension array. *BMC Immunol*. 2010;11:1-9.
- Parameswaran N, Patial S. Tumor Necrosis Factor- $\alpha$  Signaling in Macrophages Narayanan. *Adv Glob Chang Res*. 2010;20(2):87-103.
- Pangemanan DHC, Marunduh SR. Profile of Tumor Necrosis Factor-Alpha (TNF-alpha) Berdasarkan Indeks Massa Tubuh (IMT) Pada Mahasiswa Fakultas Kedokteran Unsrat Angkatan 2014. 2015;3.
- Stowe RP, Peek MK, Cutchin MP, Goodwin JS. Plasma cytokine levels in a population-based study: Relation to age and ethnicity. *J Gerontol A Biol Sci Med Sci* 2010;65 A(4):429-33.
- Yao S, Hong CC, Ruiz-Narváez EA, Evans SS, Zhu Q, Schaefer BA, et al. Genetic ancestry and population differences in levels of inflammatory cytokines in women: Role for evolutionary selection and environmental factors. *PLOS Genet*. 2018;14(6):1-17.
- Pusat B. Statistik Kota Makassar. Kota Makassar dalam Angka. 2018.
- Palmer SK, Moore LG, Young DA, Cregger B, Berman JC, Zamudio S. Altered blood pressure course during normal pregnancy and increased preeclampsia at high altitude (3100 meters) in Colorado. *Am J Obstet Gynecol* 1999;180(5):1161-8.
- Coussons-Read ME, Mazzeo RS, Whitford MH, Schmitt M, Moore LG, Zamudio S. High altitude residence during pregnancy alters cytokine and catecholamine levels. *Am J Reprod Immunol* 2002;48(5):344-54.
- Zamudio S. High-altitude hypoxia and preeclampsia. *PMC* 2019;(12):2967-77.
- Shahgheibi S, Rezaie M, Kamangar TM, Zarea S, Yousefi SR. The Effect of Season on the Prevalence of Preeclampsia. *J Clin Gynecol Obstet*. 2016;5(3):81-4.
- Jain M, Sweetey K, Shuchi J, Singh SK, Singh T. Elevated preeclampsia rates during winter with reduced sunlight dependent vitamin D production. *Integr Food Nutr Metab* 2016;2(4):207-13.